Journal of Medical Virology 61:117–124 (2000)

Seroprevalence and Seroincidence of Norwalk-Like Virus Infection Among Brazilian Infants and Children

A.H. Talal,[1,2] C.L. Moe,[1*] A.A.M. Lima,[6] K.A. Weigle,[1,3] L. Barrett,[5] S.I. Bangdiwala,[4] M.K. Estes,[7] and R.L. Guerrant[5]

[1]*Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina*
[2]*Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina*
[3]*Department of Pediatrics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina*
[4]*Department of Biostatistics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina*
[5]*Division of Geographic Medicine, University of Virginia at Charlottesville, Charlottesville, Virginia*
[6]*Clinical Research Unit, Division of Infectious Diseases, University of Ceará, Fortaleza, Brazil*
[7]*Division of Molecular Virology, Baylor College of Medicine, Houston, Texas*

To determine the importance of Norwalk-like viruses (NLVs) as pediatric pathogens in a developing country, the seroprevalence and seroincidence of this group of viruses in a cohort of children less than 4 years of age in an urban shantytown in northeastern Brazil was examined. Serum samples were collected approximately every 6 months from 135 children who were surveyed three times each week for diarrhea and vomiting. NLV IgG was measured by an enzyme immunosorbent assay (EIA) with recombinant Norwalk virus capsid protein. Overall NLV seroprevalence was 71%, and the overall NLV seroconversion rate was 0.7 seroconversions per child-year. The highest age-specific NLV seroconversion rate (0.8 seroconversions per child-year) was observed in the 13–24-month age group. For all study children, the incidence of diarrhea and vomiting was significantly greater ($P < 0.01$) during time periods spanned by serum pairs that indicated NLV seroconversion compared with time periods without NLV seroconversion. However, NLV seroconversion was not associated with gastrointestinal symptoms during the first year of life. ***J. Med. Virol. 61:117–124, 2000.***

KEY WORDS: diarrhea; viral gastroenteritis; Norwalk-like viruses; human caliciviruses

INTRODUCTION

Norwalk-like viruses (NLVs), also known as small round-structured viruses, are a group of RNA viruses in the family Caliciviridae that have been established as important causes of acute nonbacterial gastroenteritis particularly among older children and adults. These viruses are transmitted by fecally contaminated water, food, and person-to-person contact. Approximately 90% of outbreaks of acute nonbacterial gastroenteritis in adults can be attributed to NLVs [Vinje et al., 1997; Fankhauser et al., 1998]. However, the significance of NLVs as pediatric pathogens, particularly as causes of symptomatic gastroenteritis, has not been established conclusively. Until recently, NLV serologic assays have relied on antigen and antisera produced in limited supply from volunteers infected with Norwalk virus (NV). This requirement prohibited the application of these assays to large-scale seroepidemiologic studies. The production of a recombinant expressed NV capsid antigen (rNV) permitted the development of accurate serologic diagnostic tests for NLVs [Jiang et al., 1992]. The recombinant protein is comparable to the native virus as an antigenic source for serologic assays [Green et al., 1993; Monroe et al., 1993], and the rNV antigen-based EIA detects serologic responses to some other viruses related to NV [Monroe et al., 1993, Treanor et al., 1993, Jiang et al., 1996, Leite et al., 1996].

Cross-sectional studies have generally observed higher age-specific NLV seroprevalence in young children in developing countries compared with developed

Andrew Talal is currently at the Aaron Diamond AIDS Research Center, Rockefeller University, New York, NY.

Grant sponsor: National Institutes of Health; Grant number: DK07634; Grant number: DK02573; Grant number: AI U01 26512; Grant sponsor: Glaxo-Wellcome Institute for Digestive Health; Grant sponsor: General Clinical Research Center of Rockefeller University.

*Correspondence to: Christine L. Moe, Department of Epidemiology, McGavran-Greenberg Building, University of North Carolina at Chapel Hill, CB7400, Chapel Hill, NC 27599-7400. E-mail: christine_moe@unc.edu

Accepted 4 November 1999

countries [Greenberg et al., 1979; Cukor et al., 1980; Gray et al., 1993; Parker et al., 1994]. A recent seroepidemiologic study from Kuwait reported that 95% of children aged 0–7 years had antibodies to NLVs [Dimitrov et al., 1997]. Native American children in relatively isolated Amazonian communities in Brazil also have high seroprevalence of antibodies to NLVs [Gabbay et al., 1994]. Several studies have demonstrated that the degree of human contact may be an important determinant of viral transmission. In support of this hypothesis, it has been shown that (1) institutionalized children in the United States have higher age-specific NLV seroprevalence than that of age-matched controls residing with their parents [Kapikian et al., 1978], (2) children in urban areas of the United Kingdom (UK) acquire NLVs at earlier ages than children in rural regions [Gray et al., 1993], and (3) NLV seroprevalence increases at ages when children in the UK have more social contact (i.e., begin attending nursery or primary school) [Gray et al., 1993; Parker et al., 1994]. Poor hygienic conditions may also contribute to NLV infection. Among three northern Canadian communities, children in the community with the poorest quality water and sanitation had the highest incidence of infection with NLVs [Gurwith et al., 1983].

On the basis of these studies, we hypothesized that infants and young children in an area with high population density and poor living conditions may have a high incidence of NLV infection and symptomatic gastroenteritis. We report a longitudinal study of NLV seroincidence in a cohort of Brazilian infants and children aged 4–51 months living in an urban shantytown in northeastern Brazil with high population density and poor hygienic conditions. We used an enzyme immunosorbent assay (EIA) with rNV capsid antigen to determine the seroprevalence and seroincidence of NLVs and evaluated the association between viral infection and symptomatic gastroenteritis.

MATERIALS AND METHODS

Study Design

Sera were collected from children enrolled in a 4-year prospective study of the epidemiology, predisposing factors, etiology, and pathogenesis of diarrhea in infants and children living in an urban shantytown (*favela*) in Northeastern Brazil. A cohort of infants from two urban favelas within 1 mile of each other was followed from birth until 4 years of age, or until the end of the study. Prospective surveillance commenced on August 1, 1989 and terminated on April 30, 1993. All pregnancies that occurred among the 250 families in the study area were eligible for inclusion in the cohort. Overall, there were 215 pregnancies during the study period that resulted in 211 live births. A total of 186 children completed the study. Twenty-five children were excluded from the cohort for the following reasons: the families of nine children relocated before the completion of the study, eight children were placed in other families at an early age, six children prematurely withdrew from the study, one child died in infancy, and one was lost to follow-up.

Study participants were visited three times per week in their homes by a trained interviewer, a community resident, who recorded detailed information on study participants' symptoms (vomiting and diarrhea) and demographic characteristics. Serum samples were collected from each study participant approximately every 6 months.

Because previous investigators have described transplacental transmission of maternal NLV antibodies that are no longer detectable after children reach 4 months of age, we did not examine serum samples obtained before this age [Parker et al., 1994]. Four hundred fifty-two serum samples from 141 children were available for testing. Six children (32 samples) were excluded from the analysis because of high background absorbance (>0.15) in the EIA. Therefore, a total of 420 samples from 135 children were included in the seroprevalence analyses. Forty children only had one serum sample collected during the study period, and they were not included in the subsequent seroincidence analysis. A total of 285 samples from 95 children were used to examine NLV seroincidence and the relationship between NLV seroconversion and symptoms of gastroenteritis.

Laboratory Methods

NLV IgG antibodies were measured using an enzyme immunosorbent assay (EIA) as previously described [Monroe et al., 1993]. In addition to detecting NV IgG, the EIA also detects IgG to some other NLVs [Monroe et al., 1993]. Briefly, polyvinylchloride microtiter plates (Dynatech Laboratories, Chantilly, VA) were coated with either 1 μg of rNV antigen per ml in 0.01 M phosphate-buffered saline (PBS) (antigen positive) or PBS alone (antigen negative) and were incubated with 5% Blotto. After an overnight blocking reaction, sera were initially screened in duplicate at a 1:400 dilution in PBS-1% non-fat dry milk. Subsequent incubations included alkaline phosphatase-conjugated goat anti-human immunoglobulin G (IgG) (Kirkegaard & Perry, Gaithersburg, MD) diluted in PBS-1% nonfat dry milk, and p-nitrophenylphosphate (Sigma Chemical Co., St. Louis, MO) in 0.95M diethanolamine buffer (pH 9.8). After a 3-hr incubation, optical density (OD) values were determined at 410- and 650-nm wavelength. Net absorbance (P-N) was calculated as the mean value in the antigen-positive wells (P) minus the mean value in the antigen-negative wells (N) and was compared with the absorbance of a NLV reference serum. The reference serum was obtained from a patient involved in a 1978 outbreak of Norwalk virus gastroenteritis at a nursing home [Kapikian et al., 1978]. Serial dilutions of the reference serum were assayed on each plate. The net absorbance was used to construct a dose-response curve for the reference serum using a four-parameter logistic-log method (Quatro-Pro (Version 4.0), Borland, CA). The resulting parameters were then used to ex-

trapolate antibody units for each of the test sera, and the extrapolated units were multiplied by the appropriate dilution factor to calculate the total NLV IgG units in the test serum [Monroe et al., 1993]. NLV IgG units are based on an assigned concentration of total NLV IgG units in the reference serum [Monroe et al., 1993].

After the initial screening at 1:400, samples that had a reading in the antigen-negative well of >0.15 or samples that gave P-N values above the upper 2.5% of the standard curve were retested at 1:2,000. Samples that continued to have P-N values above the upper 2.5% were retested at 1:6,000. At the 1:6,000 dilution, four samples had P-N values above the upper 2.5% of the standard curve and were assigned a value at the upper limit of detection of the assay.

Statistical Analyses

Serum samples with a net absorbance of >0.1 were considered positive. A seroconversion was defined as a fourfold or greater increase in IgG units between two serum samples obtained from the same individual at least 4 weeks apart. We calculated the age-specific NLV seroprevalence for children aged 4–51 months as the proportion of seropositive children at each age interval divided by the total number of children of this age category tested. Because the number of serum samples per individual and the time intervals between sample collection were not uniform, we calculated the incidence density rate of NLV infection for all children who had one or more pairs of serum samples. We defined the incidence density rate (IDR) for NLV seroconversion as the number of seroconversions divided by the total follow-up time during which serum samples were collected, i.e., total number of child-years “at risk” of seroconversion. The time of seroconversion was defined as the midpoint between the collection of two sera samples when a fourfold or greater increase in NLV antibody titer was observed. The time at risk of each seroconversion was calculated for each child by adding the number of observation days in each age category up to the midpoint of the age category when the seroconversion occurred. The time from the midpoint to the end of the age category was counted as time at risk of the subsequent seroconversion. If a child did not seroconvert during a specific age category, the time at risk was counted toward seroconversion in a subsequent age category.

In order to evaluate the duration of NLV IgG persistence after an NLV infection in the absence of reinfection, we used the Kaplan-Meier method to analyze the decrease in NLV IgG antibody titer over time [Kaplan and Meier, 1958]. Children were eligible for this analysis if (1) they were seropositive on at least one occasion, and (2) the seropositive sample was followed by at least one additional sample. For the Kaplan-Meier calculations, the event of interest was a decline in the anti-NLV IgG titer. Individuals were censored if they had a fourfold or greater increase in titer in the next serum sample (indicating seroconversion) or if the last serum specimen obtained from the study subject showed no decrease in antibody titer. Observation time commenced with the first positive sample. The event or censoring date was defined as the midpoint between the sample that indicated the event or the censoring and the sample immediately preceding it. The time to the event or censoring was calculated for each study subject by adding the number of days from the first positive sample until the event or censoring date. All calculations were performed using STATA software (STATA Corporation (Version 4.0), College Station, TX).

Stratified analysis was used to evaluate the association between NLV seroconversion and clinical symptoms of gastroenteritis. A diarrhea episode was defined as two or more loose bowel movements per day for a minimum one-day duration. A vomiting episode was defined as at least two vomiting events per day for a minimum 1-day duration. Individual diarrhea or vomiting episodes were separated by a minimum of 2 days when the study subject was asymptomatic. The illness incidence density rate (IIDR) was calculated for vomiting and diarrhea by dividing the total number of days or episodes of vomiting or diarrhea by the total number of child-years of observation. We evaluated the association between NLV seroconversion and clinical symptoms by comparing symptom rates during time intervals between serum pairs when a seroconversion was detected to symptom rates during time intervals between serum pairs with no seroconversion. In this analysis, a child could contribute some observation time to both the “seroconversion group” and the “no seroconversion group”; therefore, the comparison groups were not independent of each other.

Statistical comparisons were performed using Fisher’s exact test, the Mantel-Haenszel chi-square statistic, or the *t*-test for independent samples using STATA statistical software.

RESULTS

NLV Seroprevalence

Anti-NLV IgG was detected in 96 of 135 (71%) of the children in this cohort who had at least one serum sample collected during the first 4 years of life. In the youngest age group, children aged 4–12 months, NLV seroprevalence was 36%. By the end of the second year of life, 70% of the children had been infected with NLV. During the third and fourth years of life, the seroprevalence stayed relatively constant at >80% (Table I).

Seroincidence of NLV Infection

Our ability to examine seroincidence over time was related to the number of serum samples collected per child. Forty children (30%) had only one serum sample, 28 (21%) had two samples, 21 (15%) had three samples, and 46 (34%) had four or more serum samples collected during the study period. The mean age at the time of collection of the first serum sample was 1.0 years and increased to 2.2 years by the time the 4th serum sample was collected (Table II).

TABLE I. Age-Specific Seroprevalence of Norwalk-Like Virus IgG

Age (mo)	No. positive/ No. children tested[a]	Percent positive
4–12	43/119	36
13–24	64/92	71
25–36	47/56	84
37–48	26/30	87
49–51	3/3	100

[a]Total number of seropositive children/total number of children in age category. A child was considered seropositive in an age category if any of the serum samples collected during that age category were positive.

Of the 95 children with at least one pair of serum samples, 44(46%) had only one seroconversion (Table II). Twenty out of 67 (30%) of children with three or more serum samples seroconverted twice, and four of 46 (9%) children with four or more serum samples seroconverted three times. No child seroconverted more than three times. Because serum samples were obtained at unequal time intervals and the number of samples per child differed, the number of seroconversions per child was adjusted by the follow-up time for each child during which serum samples were collected (Table III). The overall incidence density rate (IDR) was 0.7 NLV seroconversions per child-year of observation. Analyzing the total seroconversion rate by age, the highest IDR occurred among aged children 13–24 months with 0.8 seroconversions per child-year of observation. The age-specific seroincidence decreased in the subsequent age intervals. For first seroconversions, the IDR was also highest in the 13- to 24-month age category with 1.3 seroconversions per child-year. The second highest age-specific IDR for first seroconversion occurred during the 25- to 36-month age interval with a rate of 1.0 seroconversions per child-year. No fourth seroconversions were observed in 5.6 child-years of follow-up of children with documented third seroconversions.

Duration of Anti-NLV IgG

To determine the persistence of anti-NLV IgG in the absence of reinfection, we used the Kaplan-Meier method to examine the decline in antibody titer over time [Kaplan and Meier, 1958]. Of the 95 children with at least one pair of serum samples, 66 had at least one positive sample and met the criteria for inclusion in this analysis. During the study period, 42 (64%) of these children had a decline in antibody titer after their first positive sample. Nineteen of these individuals became seronegative, but only 15 (22%) of these 66 children remained seronegative until the end of the study. The median time until a decrease in antibody titer occurred was 158 days.

Incidence of Diarrhea and Vomiting

Children in this cohort experienced frequent episodes of diarrhea and vomiting. We first compared diarrhea and vomiting rates between study children with two or more serum samples to the illness rates in children with no serum samples and found no difference. The subsequent analyses were restricted to the 95 study children with two or more serum samples (Table IVA and IVB). Overall, there were 5.0 diarrheal episodes and 6.2 vomiting episodes per child-year of observation. The diarrhea illness incidence density rate (IIDR) was 5.0 episodes per child-year of observation in the 4- to 12-month age group, peaked at 6.4 episodes per child-year in the 13- to 24-month-old age group, and declined to 1.7 episodes per child-year of observation in the oldest age group (37–48 months) (Table IVA). The pattern observed for the vomiting IIDRs closely followed that of the diarrhea IIDRs with the highest rate of vomiting observed in the 13- to 24-month age group (8.7 episodes per child-year of observation) and then declining in older age groups (Table IVB). The IIDRs for diarrhea and vomiting days were similar to the rates of diarrhea and vomiting episodes. Overall, these children experienced 26.0 diarrhea days and 30.1 vomiting days per child-year of observation. The rates of diarrhea and vomiting days peaked in children 13–24 months of age (35.8 and 43.0 days per child-year of observation, respectively) and then declined in the older age groups (Tables IVA and IVB).

Association Between Clinical Symptoms and NLV Infection

Overall diarrhea incidence was significantly greater, an excess of 1.0 episode and 7.6 days per child-year of observation, during time intervals when children seroconverted to NLV compared with times when children did not seroconvert ($P = 0.004$ and $P < 0.001$, respectively)(Table IVA). The overall diarrhea rates were 5.6 episodes and 30.8 days per child-year of observation time with NLV seroconversion compared with 4.6 episodes and 23.2 days per child-year of observation time without seroconversion. Among children in the 4- to 12-month age category, the incidence of diarrhea was similar for children who had NLV infections and children who did not seroconvert in the first year of life (4.2 vs. 5.4 episodes and 27.5 vs. 26.8 days). However, there were significantly higher rates of diarrhea among children who seroconverted in the 13-24 month age category compared with the children in this age group who did not seroconvert (7.2 vs. 5.8 episodes [$P = 0.039$] and 38.8 vs. 33.4 days [$P < 0.001$] per child-year of observation, respectively). Differences in diarrhea rates between children who seroconverted and children who did not seroconvert were less striking in the older age groups. Increased incidence of vomiting was also associated with NLV seroconversion. Overall, there were significantly more vomiting episodes (1.6) and days (5.8) per child-year of observation among children who seroconverted compared with those who did not seroconvert ($P < 0.001$). However, in the first year of life, children who did not have NLV infections actually experienced higher vomiting rates (7.2 episodes and 34.6 days per child-year of observation) compared with children who did seroconvert in this age group (5.6 episodes and 24.3 days per child year of observation).

TABLE II. Number of Norwalk-like Virus Seroconversions per Child by Number of Samples

	Children with 1 serum sample	Children with 2 serum samples	Children with 3 serum samples	Children with ≥4 serum samples	Total
N[a] (%)	40 (30%)	28 (21%)	21 (15%)	46 (34%)	
Mean age (range) at time of serum collection (yrs):	1.0 (0.4, 3.9)	1.3 (0.5, 3.5)	1.8 (0.7, 3.1)	2.2 (1.0, 3.6)	
No. (%) seroconverted:					
Once	NA	14/28 (50)	6/21 (29)	24/46 (52)	44/95 (46)
Twice	NA	NA	5/21 (24)	15/46 (33)	20/67 (30)
Thrice	NA	NA	NA	4/46 (9)	4/46 (9)

NA, not applicable.
[a]Total number of children in each category.

TABLE III. Incidence Density Rates (IDR) of Norwalk-like Viral Infections by Age for 95 Study Children

	1st seroconversion		2nd seroconversion		3rd seroconversion		Total seroconversions[c]	
Age (mo)	No. positive/ time at risk[a]	IDR[b]	No. positive/ time at risk[a]	IDR[b]	No. positive/ time at risk[a]	IDR[b]	No. positive/ time at risk[a]	IDR[b]
4–12	23/26.7	0.9	0/4.9	0	0	0	23/31.6	0.7
13–24	36/27.2	1.3	9/26.5	0.3	1/3.1	0.3	46/56.8	0.8
25–36	8/7.8	1.0	12/28.0	0.1	3/7.1	0.4	23/42.9	0.5
37–48	0/2.0	0	3/6.0	0.5	1/4.2	0.2	4/12.2	0.3
Total:	67/63.7	1.1	24/65.4	0.4	5/14.4	0.3	96/143.6	0.7

[a]Number of seroconversions/total number of child-years of observation time during which serum samples were collected.
[b]Incidence density rate = NV seroconversions per child-year of observation.
[c]Total number of NV seroconversions/total number of child-years of observation time with serum collection.

Among older children, the vomiting rates were consistently higher among the children who seroconverted compared with those who did not seroconvert, but the differences in the age-specific vomiting rates between the two groups of children were not statistically significant.

DISCUSSION

Many studies have reported that a high percentage of children in developing countries have antibodies to NLVs [Greenberg et al., 1979; Echeverria et al., 1983; Ryder et al., 1985; Cukor et al., 1980; Black et al., 1982; Gabbay et al., 1994; Jiang et al., 1995b], and four studies have examined NLV seroincidence in young children in rural Bangladesh, rural Panama, Canada, and Finland [Black et al., 1982; Ryder et al., 1985; Gurwith et al., 1983; Lew et al., 1994], but the impact of NLV infection on child health is still largely unknown. This study reports an association between NLV seroconversion and vomiting in young children and evaluates NLV seroincidence among urban children in a developing country. Our study population experienced a heavy burden of gastrointestinal illness (26 diarrhea days and 30 vomiting days per child-year of observation), and we observed significantly higher overall incidence of vomiting and diarrhea episodes and days in children with a serologic response to NLV compared with children with no NLV seroconversion. The association was strongest among children aged 13–24 months when both gastrointestinal symptom rates and NLV seroincidence peaked and when most of our serum samples were collected. However, in children less than 1 year of age, NLV seroconversion was not associated with gastrointestinal symptoms, and higher vomiting rates were observed among children who did not have NLV seroconversion. It is likely that other enteric infections, particularly rotavirus, are the dominant cause of vomiting in the first year of life. In order to examine this hypothesis, we looked at results on fecal specimens from symptomatic children less than 1 year of age and from all NLV seropositive children. Ten (7%) of 146 fecal specimens collected from study children less than 1 year of age with diarrhea were positive for rotavirus. Analyses of fecal specimens, obtained from 94 NLV seropositive study subjects at the time of or before NLV seropositivity was detected, indicated that 34 children had pathogen-negative stool samples and the remaining 60 had various enteric pathogens, including: infection by genogroup I and genogroup II NLVs in 22 children [Parks et al., 1999], rotavirus (2), torovirus (2) [Koopmans et al., 1997], pathogenic *Escherichia coli* and other bacterial pathogens (56), and *Cryptosporidium*, or *Giardia* (12) (data not shown). These findings confirm that NLVs were one of many enteric pathogens responsible for the gastroenteritis symptoms observed in this cohort.

Other investigators have also examined the association between symptoms of gastroenteritis and NLV seroconversion. Black et al. (1982) reported significantly higher incidence of diarrhea episodes in which no pathogen could be identified among rural Bangladeshi children aged 2–49 months who seroconverted to NLV compared with children who did not seroconvert. However, Lew et al. (1994) did not find an association between NLV infection and diarrhea or vomiting in Finnish children less than 2 years of age. In our study, the

TABLE IV. Association Between Clinical Symptom Rates and NLV Seroconversion by Age for 95 Study Children With Two or More Serum Samples

	Observation time with seroconversion					Observation time without seroconversion					All children	
Age (mo)	Child-days observation	No. of episodes	Episode rate[a]	No. of days	Day rate[a]	Child-days observation	No. of episodes	Episode rate[a]	No. of days	Day rate[a]	Episode IIDR	Day IIDR
					A. Diarrheal symptoms							
4–12	1,491	17	4.2	112	27.5	3,112	46	5.4	228	26.8	5.0	27.6
13–24	10,007	197	7.2*	1,063	38.8***	12,761	204	5.8	1,168	33.4	6.4	35.8
25–36	7,853	101	4.7	576	26.8	12,921	173	4.9	832	23.5	4.8	24.7
37–48	1,592	8	1.8	16	3.7	8,003	38	1.7	109	5.0	1.7	4.8
Total:	20,943	323	5.6**	1,767	30.8†	36,797	461	4.6	2,337	23.2	5.0	26.0
IIDR diff[b]			1.0		7.6							
IIDR ratio[c]			1.2		1.3							
					B. Vomiting symptoms							
4–12	1,491	23	5.6	99	24.3	3,112	61	7.2	294	34.6‡‡	6.7	31.2
13–24	10,007	260	9.5	1,218	44.4	12,761	282	8.1	1,467	42.0	8.7	43.0
25–36	7,853	119	5.5	635	29.5	12,921	179	5.1	995	28.1	5.2	28.6
37–48	1,592	12	2.8	32	7.3	8,003	42	1.9	148	6.8	2.1	6.8
Total:	20,943	414	7.2††	1984	34.6‡	36,797	564	5.6	2,904	28.8	6.2	30.1
IIDR diff[d]			1.6		5.8							
IIDR ratio[e]			1.3		1.2							

[a]IIDR, illness incidence density rate of diarrhea or vomiting per child-year of observation.

[b]IIDR diff, total diarrhea illness incidence density rate for observation time with seroconversion minus total diarrhea illness incidence density rate for observation time without seroconversion.

[c]IIDR ratio, total diarrhea illness incidence density rate for observation time with seroconversion divided by total diarrhea illness incidence density rate for observation time without seroconversion.

[d]IIDR diff, total vomiting illness incidence density rate for observation time with seroconversion minus total vomiting illness incidence density rate for observation time without seroconversion.

[e]IIDR ratio, total vomiting illness incidence density rate for observation time with seroconversion divided by total vomiting illness incidence density rate for observation time without seroconversion.

*$P = 0.039$, Fisher's exact test comparing diarrhea episodes for time intervals with seroconversion to time intervals without seroconversion among children aged 13–24 months.

**$P = 0.004$, Fisher's exact test comparing diarrhea episodes for time intervals with seroconversion to time intervals without seroconversion among all study children.

***$P < 0.001$, Fisher's exact test comparing diarrhea days for time intervals with seroconversion to time intervals without seroconversion among children aged 13–24 months.

†$P < 0.001$, Fisher's exact test comparing diarrhea days for time intervals with seroconversion to time intervals without seroconversion among all study children.

††$P < 0.001$, Fisher's exact test comparing vomiting episodes for time intervals with seroconversion to time intervals without seroconversion among all study children.

‡$P < 0.001$, Fisher's exact test comparing vomiting days for time intervals with seroconversion to time intervals without seroconversion among all study children.

‡‡$P = 0.002$, Fisher's exact test comparing vomiting days for time intervals with seroconversion to time intervals without seroconversion among children aged 4–12 months.

intense active surveillance for gastrointestinal symptoms, the sensitivity of the rNV EIA, our quantitative methods for estimating IgG titer and our incidence density analytical approach contributed to the conclusion of a significant association between NLV seroconversion and symptoms of gastroenteritis.

The overall IDR of 0.7 NLV seroconversions per child-year in children less than 4 years of age that we observed is similar to that reported by Ryder et al. (1985) in young children in rural Panama (0.63) but is higher than the NLV seroincidence rates reported by three other longitudinal studies of NLV infection in children in Canada (<0.001–0.19), Finland (0.27) and rural Bangladesh (0.29) [Gurwith et al., 1983; Lew et al., 1994; Black et al., 1982]. Yet, our estimate of NLV seroincidence is likely to be an underestimate of the total burden of human calicivirus infection in this study population. Some NLV infections may have been missed because of the long time span between serum collection from some study children. Our analysis of the duration of anti-NLV IgG indicated that antibody titer decreases after a median of 158 days in the absence of reinfection. This estimate is limited because we do not know how long after NLV infection the positive serum samples were obtained or how long after an individual became seronegative a negative serum sample was collected. However, our data does suggest that anti-NLV IgG persists long enough to be a useful marker of recent NLV infection in comparison to a previously taken serum. Serologic responses to infections with genogroup II NLVs and Sapporo-like viruses that are more distantly related to NV (genogroup I) may not have been detected by our rNV-based EIA. Several investigators have shown that there is limited cross-reactivity in the serological response of volunteers and outbreak cases infected with genogroup II NLVs to the rNV antigen [Monroe et al., 1993; Treanor et al., 1993; Jiang et al., 1996; Leite et al., 1996; Noel et al., 1997]. Our observation of high seroincidence and seroprevalence and the fact that most of the seropositive children in this cohort remained seropositive for the duration of the study indicates frequent NLV infection and reinfection. In this cohort, anti-NLV IgG did not protect against reinfection from another NLV, even of the same genogroup [Parks et al., 1999].

Previous seroepidemiologic studies of NLV have suggested that local sanitation conditions and the degree of human contact may be important determinants of NLV transmission [Parker et al., 1994; Gray et al., 1993; Gurwith et al., 1983; Gabbay et al., 1994]. In developing countries, children become infected with NLV early in life, suggesting early exposure to fecal contamination at home. In our study population, 36% of the children in the youngest age group (4–12 months) had NLV antibodies. In this population, supplemental food and water are introduced during the first month of life and may well serve as the primary vehicles of NLV transmission. Poor sanitary conditions (fewer than 50% of the households had access to piped water and less than 10% of study households had flush toilets) may have contributed to the high NLV seroprevalence and seroincidence we observed.

On the basis of the high NLV seroprevalence they observed among young children in several remote Amazonian communities, Gabbay et al (1994) speculated that NLVs may be sporadically introduced by urban migrants into these communities and then spread rapidly under poor hygienic conditions. Our findings support this hypothesis and indicate that young children in an urban shantytown in a developing country experience frequent, symptomatic NLV infections. While NLVs are well recognized as a cause of epidemic acute gastroenteritis in adults and older children in developed countries, their role in endemic pediatric gastroenteritis in developing countries has been underestimated. Given the global magnitude and impact of pediatric gastroenteritis, further investigations should characterize the epidemiology of these viruses in pediatric populations and identify effective public health measures to prevent these infections.

ACKNOWLEDGMENTS

We are grateful to Dr. Steve Monroe, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, for providing positive control serum and sharing his data analysis methods. We also thank Fan-Chen Tseng for her assistance in establishing the antibody assay in the laboratory and Joseph Galanko and Chulaluk Komoltri for assistance with statistical computations.

REFERENCES

Black RE, Greenberg HB, Kapikian AZ, Brown KH, Becker S. 1982. Acquisition of serum antibody to Norwalk virus and rotavirus and relation to diarrhea in a longitudinal study of young children in rural Bangladesh. J Infect Dis 145:483–489.

Cukor G, Blacklow NR, Echeverria P, Bedigian MK, Puruggan H, Basaca-Sevilla V. 1980. Comparative study of the acquisition of antibody to Norwalk virus in pediatric populations. Infect Immun 29:822–823.

Dimitrov DH, Dashti SAH, Ball JM, Bishbishi E, Alsaeid K, Jiang X, Estes MK. 1997. Prevalence of antibodies to Human Caliciviruses (HuCVs) in Kuwait established by ELISA using baculovirus-expressed capsid antigens representing two genogroups of HuCVs. J Med Virol 51:115–118.

Echeverria P, Burke DS, Blacklow NR, Cukor G, Charoenkul C, Yanggratoke S. 1983. Age-specific prevalence of antibody to rotavirus, *Escherichia coli* heat-labile enterotoxin, Norwalk virus, and hepatitis A virus in a rural community in Thailand. J Clin Microbiol 17:923–925.

Fankhauser RL, Noel JS, Monroe SS, Ando T, Glass RI. 1998. Molecular epidemiology of "Norwalk-like Viruses" in outbreaks of gastroenteritis in the United States. J Infect Dis 178:1571–1578.

Gabbay YB, Glass RI, Monroe SS. 1994. Prevalence of antibodies to Norwalk virus among Amerindians in isolated Amazonian communities. Am J Epidemiol 139:728–733.

Gray JJ, Jiang X, Morgan-Carner P, Desselberger U, Estes MK. 1993. Prevalence of antibodies to Norwalk virus in England: detection of enzyme-linked immunosorbent assay using baculovirus-expressed Norwalk virus capsid antigen. J Clin Microbiol 31:1022–1025.

Green KY, Lew JF, Jiang X, Kapikian AZ, Estes MK. 1993. Comparison of the reactivities of baculovirus-expressed recombinant Norwalk virus capsid antigen with those of the native Norwalk virus antigen in serologic assays and some epidemiologic observations. J Clin Microbiol 31:2185–2191.

Greenberg HB, Valdesuso J, Kapikian AZ, Chanock RM, Wyatt RG, Szmuness W, Larrick J, Kaplan J, Gilman RH, Sack DA. 1979.

Prevalence of antibody to the Norwalk virus in various countries. Infect Immun 26:270–273.

Gurwith M, Wenman W, Gurwith D, Brunton J, Feltham S. Greenberg, HB. 1983. Diarrhea among infants and young children in Canada: a longitudinal study in three northern communities. J Infect Dis 147:685–692.

Jiang X, Wang M, Graham DY, Estes MK. 1992. Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. J Virol 66:6527–6532.

Jiang X, Matson DO, Ruiz-Palacios GM, Hu J, Treanor J, Pickering LK. 1995. Expression, self-assembly, and antigenicity of a Snow Mountain agent-like calicivirus capsid protein. J Clin Microbiol 33:1452–1455.

Jiang X, Matson DO, Velazquez FR, Zhong WM, Hu J, Ruiz-Palacios G, Pickering LK. 1995. A study of Norwalk-related viruses in Mexican children. J Med Virol 47:309–316.

Jiang X, Matson DO, Cubitt WD, Estes MK. 1996. Genetic and antigenic diversity of human caliciviruses (HuCVs) using RT-PCR and new EIAs. Arch Virol 12(suppl):251–262.

Kapikian AZ, Greenberg HB, Cline WL, Kalica AR, Wyatt RG, James HD Jr, Lloyd NL, Chanock RM, Ryder RW, Kim HW. 1978. Prevalence of antibody to Norwalk agent by a newly developed immune adherence hemagglutination assay. J Med Virol 2:281–294.

Kaplan EL, Meier P. 1958. Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457–481.

Kaplan JE, Gary GW, Baron RC, Singh N, Schonberger LB, Feldman R, Greenberg HB. 1982. Epidemiology of Norwalk gastroenteritis and the role of Norwalk virus in outbreaks of acute nonbacterial gastroenteritis. Ann Intern Med 96:756–761.

Koopmans MPG, Goosen ESM, Lima AAM, McAuliffe IT, Nataro JP, Barrett LJ, Glass RI, Guerrant RL. 1997. Association of torovirus with acute and persistent diarrhea in children. Pediatr Infect Dis J 16:504–507.

Leite JP, Ando T, Noel JS, Jiang B, Humphrey CD, Lew JF, Green KY, Glass RI, Monroe SS. 1996. Characterization of Toronto virus capsid protein expressed in baculovirus. Arch Virol 141:865–875.

Lew JF, Valdesuso J, Vesikari T, Kapikian AZ, Jiang X, Estes MK, Green KY. 1994. Detection of Norwalk virus or Norwalk-like virus infections in Finnish infants and young children. J Infect Dis 69: 1364–1367.

Monroe SS, Stine SE, Jiang X, Estes MK, Glass RI. 1993. Detection of antibody to recombinant Norwalk virus antigen in specimens from outbreaks of gastroenteritis. J Clin Microbiol 31:2866–2872.

Noel JS, Ando T, Leite JP, Green KY, Dingle KE, Estes MK, Seto Y, Monroe SS, Glass RI. 1997. Correlation of patient immune responses with genetically characterized small round-structured viruses involved in outbreaks of nonbacterial gastroenteritis in the United States, 1990 to 1995. J Med Virol 53:372–383.

Parker S, Cubitt D, Jiang JX, Estes MK. 1994. Seroprevalence studies using a recombinant Norwalk virus protein enzyme immunoassay. J Med Virol 42:146–150.

Parks C, Moe C, Rhodes D, Lima A, Barrett L, Tseng F, Baric R, Talal A, Guerrant RL. 1999. Genetic diversity of "Norwalk-like viruses" (NLVs): pediatric infections in a Brazilian shantytown. J Med Virol 58:426–434.

Ryder RW, Singh N, Reeves WC, Kapikian AZ, Greenberg HB. 1985. Evidence of immunity induced by naturally acquired Rotavirus and Norwalk virus infection on two remote Panamanian Islands. J Infect Dis 1:99–105.

Treanor JJ, Jiang X, Madore HP, Estes MK. 1993. Subclass-specific serum antibody responses to recombinant Norwalk virus capsid antigen (rNV) in adults infected with Norwalk, Snow Mountain, or Hawaii Virus. J Clin Microbiol 31:1630–1634.

Vinje J, Altens SA, Koopmans MPG. 1997. The incidence and genetic variability of small round structured viruses in outbreaks of gastroenteritis in The Netherlands. J Infect Dis 176:1374–1378.